ANTINEUTROPHIL CYTOPLASMATIC AUTOANTIBODIES
IN ULCERATIVE COLITIS

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ABSTRACT - Antineutrophil cytoplasmatic antibodies (ANCA) were detected in patients with certain autoimmune and vascular diseases such as Wegener's granulomatosis, Polyarteritis nodosa, and systemic lupus erythematosus. Indirect immunofluorescence (IF) technique was employed to detect these autoantibodies. ANCA have been recently detected in some forms of inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD), and primary sclerosing cholangitis (PSC). By IF method, two general patterns of ANCA were seen: a cytoplasmic (c-ANCA) and perinuclear form (p-ANCA). In this study we evaluated the presence of ANCA in 52 UC patients and 60 matched normal control group by IF technique. Results showed that all control group were ANCA negative, but 8% of patients had ANCA, and most cases (70%) had c-ANCA. The obtained results also revealed that there was no relationship between ANCA and disease activity.


Key Words: Antineutrophil cytoplasmatic antibodies, ulcerative colitis

INTRODUCTION

Antineutrophil cytoplasmatic antibodies (ANCA) are autoantibodies directed against endosomal enzymes of human neutrophils and monocytes. These antibodies have been detected in various forms of vasculitis, including segmental necrotizing glomerulonephritis, Wegener's granulomatosis (WG), and microscopic polyarteritis (MPA). ANCA have been recently detected in some forms of inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD), and primary sclerosing cholangitis (PSC) using indirect immunofluorescence and fixed granulocyte (GFA) (34, 35). Two major staining patterns can be distinguished for indirect immunofluorescence (IF), a cytoplasmic pattern (c-ANCA), and a perinuclear rim (p-ANCA) (36). The main target antigen associated with c-ANCA is proteinase-3 (PR-3) and for p-ANCA is myeloperoxidase (MPO). In the present study, we examined the presence of ANCA in 52 UC patients and 60 normal control group by IF technique. Relationship between ANCA and disease activity, site of colon involvement and lesion extent were the other goals of this study.

MATERIALS AND METHODS

52 UC patients (31 female and 21 male) from different provinces referred to gastroenterology section of Shariat Hospital, Tehran were selected. 60 healthy adult volunteers were studied as control group. The subjects sera were screened for antineutrophil cytoplasmatic antibodies (ANCA) and antinuclear activity (ANA) by indirect immunofluorescence technique (IF).

Presence of ANCA in undiluted serum and detection of ANA in serum dilution greater than 1:40 were our criteria to consider a patient's serum as positive. P-ANCA positive subjects with positive ANA were excluded and considered as ANCA negative.

Statistical analysis (Chi-square and T-test) was done to determine the correlation of ANCA positivity and disease activity and the significance of different types of ANCA.

RESULTS

In this study the presence of ANCA in 52 patients with ulcerative colitis (31 female 14.58 years old and 21 male 16.66 years old ) was evaluated. 60 healthy matched volunteers were considered as control group. The patients were classified into 4 groups (active mild, moderate and severe) according to the clinical symptoms (frequency of diarrhea) in the patients (table 1). 40 out of 52 UC cases (76%) were in the inactive form and the 12 remittced (24%) had active form of the disease.

Table 1. Presence of ANCA in ulcerative colitis patients relevant to disease activity

<table>
<thead>
<tr>
<th>Disease Activity</th>
<th>ANCA-</th>
<th>P-ANCA</th>
<th>C-ANCA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>60</td>
<td>3</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>Active cases</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Mild cases</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Moderate cases</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Severe cases</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>9</td>
<td>21</td>
<td>59</td>
</tr>
</tbody>
</table>

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ANCA in ulcerative colitis

According to the site of colon involvement and ulcer extent the patients were also classified into 6 groups (table 2). The majority of cases had pancolitis (16 cases) and left sided colitis (15 cases). Table 1 and 2 show that 30 (58%) UC cases have ANCA out of whom 21 of them (70%) are 1- ANCA type and 9 reminders (30%) P-ANCA positive. 23 (76%) out of 30 ANCA positive cases were in the active form and 7 (24%) were in the inactive form of the disease. As indicated in table 2, 11 out of 16 pancolitis cases and 9 out of 15 left sided colitis were ANCA positive.

Statistical analysis of the results revealed that there was no significant correlation between ANCA presence, disease activity and site of colon involvement.

Table 2. Prevalence of antineutrophil cytoplasmic antibodies (ANCA) in patients with different kinds of ulcerative colitis.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total ANCA+ ANCA- P-ANCA+ P-ANCA-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancolitis</td>
<td>16 11 5 2 9</td>
</tr>
<tr>
<td>Subtotal</td>
<td>5 2 3 1 2</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>6 2 4 1 1</td>
</tr>
<tr>
<td>Left sided</td>
<td>15 9 6 2 7</td>
</tr>
<tr>
<td>Proctitis</td>
<td>8 5 5 3 2</td>
</tr>
<tr>
<td>Total</td>
<td>52 39 22 9 21</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study ANCA presence in patients with UC was evaluated. The results showed that 30 out of 52 UC sera (58%) contained ANCA, while all control samples were ANCA negative. Similar findings concerning ANCA frequency in UC subjects were reported by others, Raimp JA, and colleagues (1990) have shown that 20 of 34 (59%) sera from patients with ulcerative colitis had P-ANCA (9), Brown et al (1994) found P-ANCA in 33 of 67 (49%) ulcerative colitis patients (10). Sonogami and colleagues (1990) found the clinical significance of ANCA in ulcerative colitis patients with either an indirect immunofluorescence assay or an ELISA with fixed neutrophils, 7/12 (58%) of the patients were positive for ANCA (11). The most commonly observed pattern of ANCA in UC patients in other studies were P-ANCA (10), (12,13) but Sung and colleagues (1994) in a study on Chinese patients with ulcerative colitis (N=9) indicated that 75% of subjects exhibited either P-ANCA (50%) or 1- ANCA (25%). Also, our study showed that 21 out of 30 ANCA positive patients (70%) had C-ANCA and 9 reminders (30%) had P-ANCA. In this regard, the genetic background of population, and environmental agents may be the suspected causative factors.

Alfad and colleagues (1997) in a study showed that most ANCA positive sera from IBD patients were negative for antibodies to proteinase 3 and myeloperoxidase by ELISA (3). They suggested that the autoantigens recognized by ANCA were different in patients with IBD from those with necrotizing vasculitis.

In the study conducted by Roma and colleagues none of the ANCA positive patients had antibodies to myeloperoxidase or to alpha granules which are usually found in sera of patients with ANCA associated vasculitis (13). As reported in literature it seems that the P-ANCA staining pattern of granulocytes is not restricted to anti-myeloperoxidase antibodies. More recently a study on the antigen specificity and new antigen of ANCA positive UC patients demonstrated that high mobility group (HMG) non-histone chromosomal proteins (HMG1 and HMG2) are novel target antigens of P-ANCA, HMG1 and HMG2 are distributed in the nuclei and cytoplasm of epithelial cells and act as transcription factors (16,17).

As it has been shown in tables 1 and 2, there is not any significant correlation between ANCA positivity and variables such as disease activity, site of colon involvement and lesion extent.

Treatment of the disease also had no correlation with ANCA positivity (unpublished data). The study performed by Sonogami (1990) also revealed that there was no significant correlation between the ANCA positivity and various variables, i.e. disease activity, extent of lesion , or treatment of the disease. In contrast, Henriqv (1994) in a study on 153 patient with ulcerative colitis showed that the presence of ANCA was correlated to disease activity, extent, and age of onset of the disease (13).

Our results also revealed that there was no correlation between ANCA and ANA in these patients which indicates that the immune response against neutrophil cytoplasmic antigens is independent of the response against nucleolar antigens.

Future investigations must be conducted to precisely evaluate the importance of these autoantibodies and their correlation to disease activity in inflammatory bowel disease. The type of ANCA, the target antigen of these autoantibodies and the importance of newly recognized autoantigens in UC, are the other subjects that must be considered in future studies.

REFERENCES


